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1. Introduction

- The number of new drugs that are being designed for therapy is constantly increasing. These drugs or formulations may be either new entities in the market or partial structural modifications of the existing drugs.
- The introduction of large number of newer drugs and their formulations may also lead to widespread distribution of substandard or even counterfeit drugs and their formulations in the market.
- It is necessary to find out the content of each drug either in bulk samples or its formulations to check their purity. It is also essential to know the concentration of the drug and its metabolites in body fluids after taking the dosage form during treatment.

2. Aim and objective

- There is a necessity for developing newer and efficient methods for determining these drugs in plasma samples /formulations
- Quality control and quality assurance of pharmaceutical chemicals and their formulations are essential for ensuring the availability of safe and effective drug formulations to the consumers and safeguarding the general public against the hazards of substandard drugs.
- Thus, constant development of new and improved analytical methods for accurate determination of drugs in raw materials, in pharmaceutical dosage forms and plasma samples is essential for quality control, pharmacokinetic, bioequivalence and toxicological studies.

3. Materials & Methods

List of drugs selected for the study

1. Niflumic Acid
2. Mesalamine
3. Meloxicam

4. Clopidogrel
5. Simvastatin
6. Lornoxicam
7. Naproxen
8. Diclofenac Sodium

The above mentioned drugs are selected for study. Out of which first six drugs are selected for analytical method development and validations in bulk and their formulations by using RP-HPLC [1-3].

The last three drugs are selected for bio analytical method development and validation in plasma samples using LC-MS/MS.

The details of methods are described in results and discussions part.

4. Results & discussions

The development of a stability indicating reverse phase HPLC with UV-Visible detector method for the determination of Niflumic acid using phenomenx RP-C18 (250x4.6mm, packed with Luna 5 µ) column. A mobile phase consisting of methanol: water (75:25%v/v) was employed in this study. The flow rate was kept at 1.0 ml/min and the injection volume was 20 µl. The separation was performed at ambient temperature. Eluents were monitored by UV detector set at 254 nm. The developed method was statistically validated for the linearity, precision, accuracy, robustness, specificity, LOD and LOQ. The specificity of the method was ascertained by force degradation studies by acid hydrolysis, alkali hydrolysis & degradation by oxidation. The degraded products were well resolved from the analyte peak with significant difference in their RT values.

A sensitive & selective stability indicting RP-HPLC method has been developed & validated for the analysis of Mesalamine. Based on peak purity results, obtained from the analysis of force degradation samples using described method, it can be concluded that the absence of co-eluting peak along with the main peak of Mesalamine indicated that the developed method is specific for the estimation of Mesalamine in presence of degradation products. Further the proposed RP-HPLC method has excellent sensitivity, precision and reproducibility.

Separation was achieved on Develosil ODS HG-5 RP C_{18}, (15cmx4.6mm i.d. 5µm) column in isocratic mode with mobile phase consisting of acetonitrile :phosphate buffer(pH 3.4) (60:40) with a flow rate of 1 mL/min. The detection was carried out at 268 nm. The retention time of Meloxicam was found to be 2.09 min.The method was validated as per ICH guidelines. Linearity was established for Meloxicam in the range 20 – 120 µg / ml with R^2 value 0.996. The percentage recovery of Meloxicam was found to be in the range 99.99-100.46 %. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the estimation of the drug in bulk and tablet dosage forms.

Separation was achieved on Develosil ODS HG-5 RP C_{18}, (15cmx4.6mm i.d. 5µm) column in isocratic mode with mobile phase consisting of acetonitrile :phosphate buffer(pH 2.85) (65:35) with a flow rate of 1 mL/min. The detection was carried out at 225 nm. The retention time of Clopidogrel was found to be 7.48 min.The method was validated as per ICH guidelines. Linearity was established for Clopidogrel in the range 10 – 60 µg / ml with R^2 value 0.999. The percentage recovery of Clopidogrel was found to be in the range 99.71-100.03 %.

A novel, simple and economic reverse phase high performance liquid chromatography (RP-HPLC) method has been developed for the estimation of Simvastatin in bulk and tablet dosage form with greater precision and accuracy. Separation was achieved on Develosil ODS HG-5 RP C_{18}, (150cmx4.6mm i.d. 5µm) column in isocratic mode with mobile phase consisting of acetonitrile :phosphate buffer(pH 3.0) (85:15) with a flow rate of 1 mL/min. The detection was carried out at 236 nm. The retention time of Simvastatin was found to be 5.84 min. The method was validated as per ICH guidelines.Linearity was established for Simvastatin in the range 10 – 100 µg / ml with R^2 value 0.99. The percentage recovery of Simvastatin was found to be in the range 99.19-99.67 %. The high recovery and low relative standard deviation confirm the suitability of the proposed method for the estimation of the drug in bulk and tablet dosage forms. The LOD and LOQ were found to be 0.341 and 1.023 µg/ml respectively.

A novel liquid chromatography tandem mass spectrometry method is described for the quantitative determination of lornoxicam in human K_2 EDTA plasma in positive ion mode and validated using piroxicam as internal standard (ISTD) according to linearity, selectivity, precision, recovery and various stability studies. Sample preparation was accomplished by liquid liquid extraction technique. The eluted samples were

chromatographed on ACE C_{18} (150 x 4.6 mm, 5 µ) column (Agilent Technologies) using a mobile phase consisting of HPLC grade acetonitrile:0.3% formic acid buffer (80:20 v/v) with injection volume of 15 µL and a run time of 3.0 minutes. The precursor to product ion transitions m/z 372.10 to 121.10 (Lornoxicam) and m/z 332.10 to 95.20 (Piroxicam, IS) were used for quantization. The calibration graph of lornoxicam was linear with $r^2 > 0.99$ over a concentration range of 5.086 ng/mL to 1518.325 ng/mL. CV % of intra- and inter-day precisions were found satisfactory and well within the limits. The drug was found to be stable for the studied parameters and found to be interference free for matrix effect with appreciable recovery.

A new LC-MS/MS method includes simple and single step extraction procedure using inexpensive chemicals and short run time (3.0 min) for determination of naproxen in human plasma with all validation reports is described in this work, which can be implemented for therapeutic drug monitoring.A novel liquid chromatography–tandem mass spectrometry method is described for the quantitative determination of naproxen in human K_2-EDTA plasma in negative ion mode and validated using Zidovudine as internal standard (IS). Sample preparation was accomplished by liquid-liquid extraction technique. The eluted samples were chromatographed on Zorbax Eclipse XDB phenyl 4.6 X 75 mm, 3.5 µm column (Agilent Technologies) using a mobile phase consisting of acetonitrile: 20 mM ammonium acetate (90:10 v/v).The injection volume was 15 µL and the total run time was 3.0 minutes. The method was validated for all parameters for Naproxen. The method shows selectivity and linearity over a concentration range of 500.1 ng/mL to 100028.5 ng/mL. This validation report provides the results of selectivity, Calibration standards data, precision and accuracy data (% Nominal between 90-110% and % C.V below 15%), the results of recovery (80.63%), various stabilities(Mostly stable) and carryover test(No carry over i.e. 0 %) along with all pertinent supporting documentation.

A novel liquid chromatography tandem mass spectrometry method is described for the quantitative determination of Diclofenac in human K_2 EDTA plasma in negative ion mode and validated using Zidovudine as internal standard (ISTD). Sample preparation was accomplished by solid phase extraction technique. The eluted samples were chromatographed on Zorbax XDB phenyl column (75 x 4.6 mm, 3.5 µ) ,Agilent Technologies using a mobile phase consisting of HPLC grade acetonitrile:0.2% acetic acid in HPLC water (80:20 v/v).The injection volume was 15 µL and the total run time was 2.0 minutes. The method was validated over a linear concentration range of 25.02 ng/mL to 4004.78 ng/mL for diclofenac.

The precursor to product ion transitions m/z 294.10 to 249.90 (diclofenac) and m/z 266.0 to 222.90 (zidovudine, IS) were used for quantitation. The retention times were 1.12 and 0.58 mins for diclofenac and zidovudine, respectively.

5. Conclusion

From the above findings it is clear that the proposed validated liquid chromatographic methods can be successfully implemented for the estimation of above mentioned drugs in formulations / plasma samples effectively.

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6. References

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